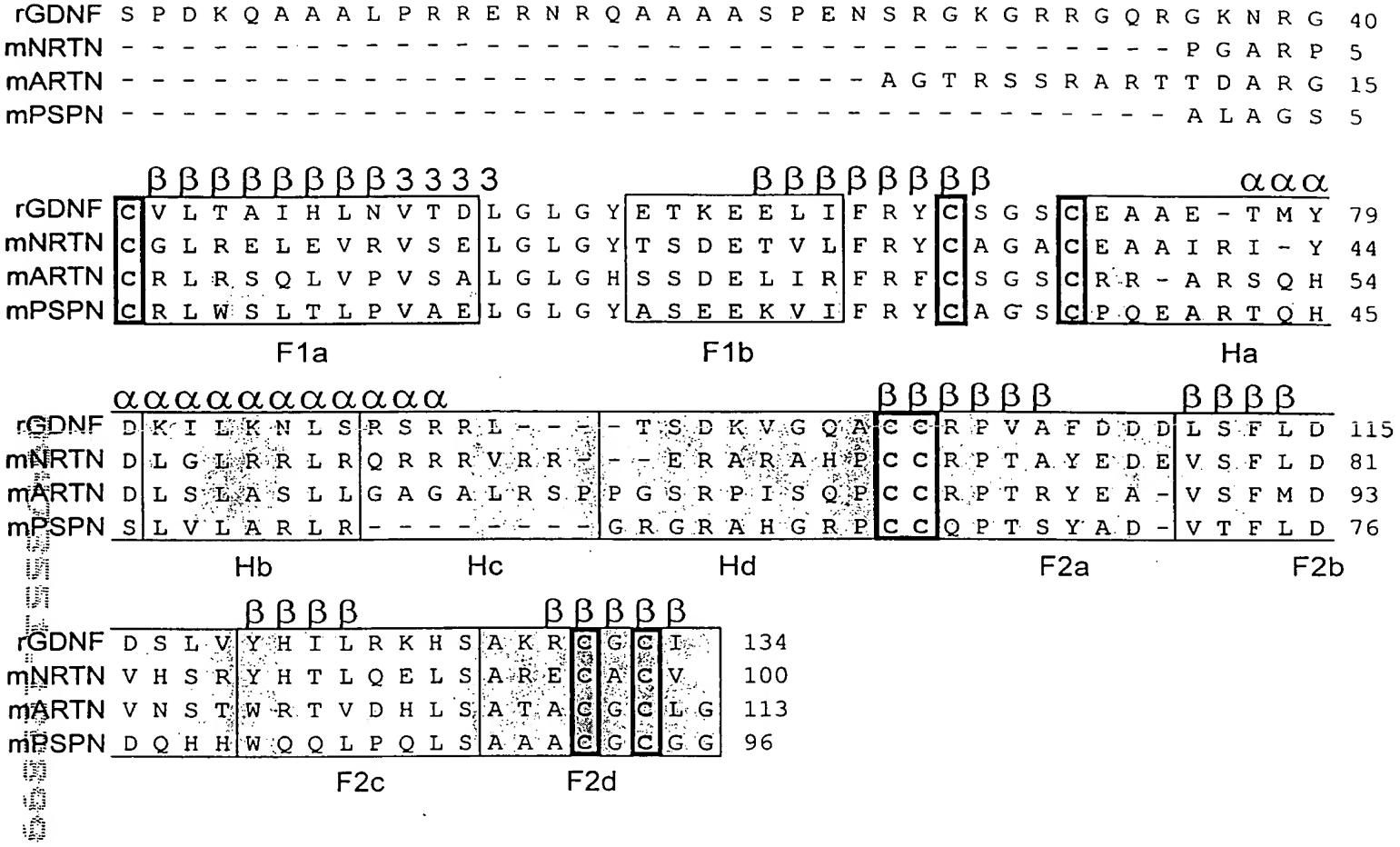

rGDNF S P D K Q A A A L P R R E R N R Q A A A A S P E N S R G K G R R G Q R G K N R G 40
mNRTN - P G A R P 5
mARTN - A G T R S S R A R T T D A R G 15
mPSPN - A L A G S 5
β β β β β β β β 3 3 3 3
β β β β β β β β
α α α
rGDNF C V L T A I H L N V T D L G L G Y E T K E E L I F R Y C S G S C E A A E - T M Y 79
mNRTN C G L R E L E V R V S E L G L G Y T S D E T V L F R Y C A G A C E A A I R I - Y 44
mARTN C R L R S Q L V P V S A L G L G H S S D E L I R F R F C S G S C R R - A R S Q H 54
mPSPN C R L W S L T L P V A E L G L G Y A S E E K V I F R Y C A G S C P Q E A R T Q H 45
F1a
F1b
Ha
α α α α α α α α α α α α α
β β β β β β
β β β β
rGDNF D K I L K N L S R S R R L - - - - T S D K V G Q A C C R P V A F D D D L S F L D 115
mNRTN D L G L R R L R Q R R R V R R - - - E R A R A H P C C R P T A Y E D E V S F L D 81
mARTN D L S L A S L L G A G A L R S P P G S R P I S Q P C C R P T R Y E A - V S F M D 93
mPSPN S L V L A R L R - - - - - - - - G R G R A H G R P C C Q P T S Y A D - V T F L D 76
Hb
Hc
Hd
F2a
F2b
β β β β
β β β β β
rGDNF D S L V Y H I L R K H S A K R C G C I 134
mNRTN V H S R Y H T L Q E L S A R E C A C V 100
mARTN V N S T W R T V D H L S A T A C G C L G 113
mPSPN D Q H H W Q Q L P Q L S A A A C G C G G 96
F2c
F2d

FIGURE 1

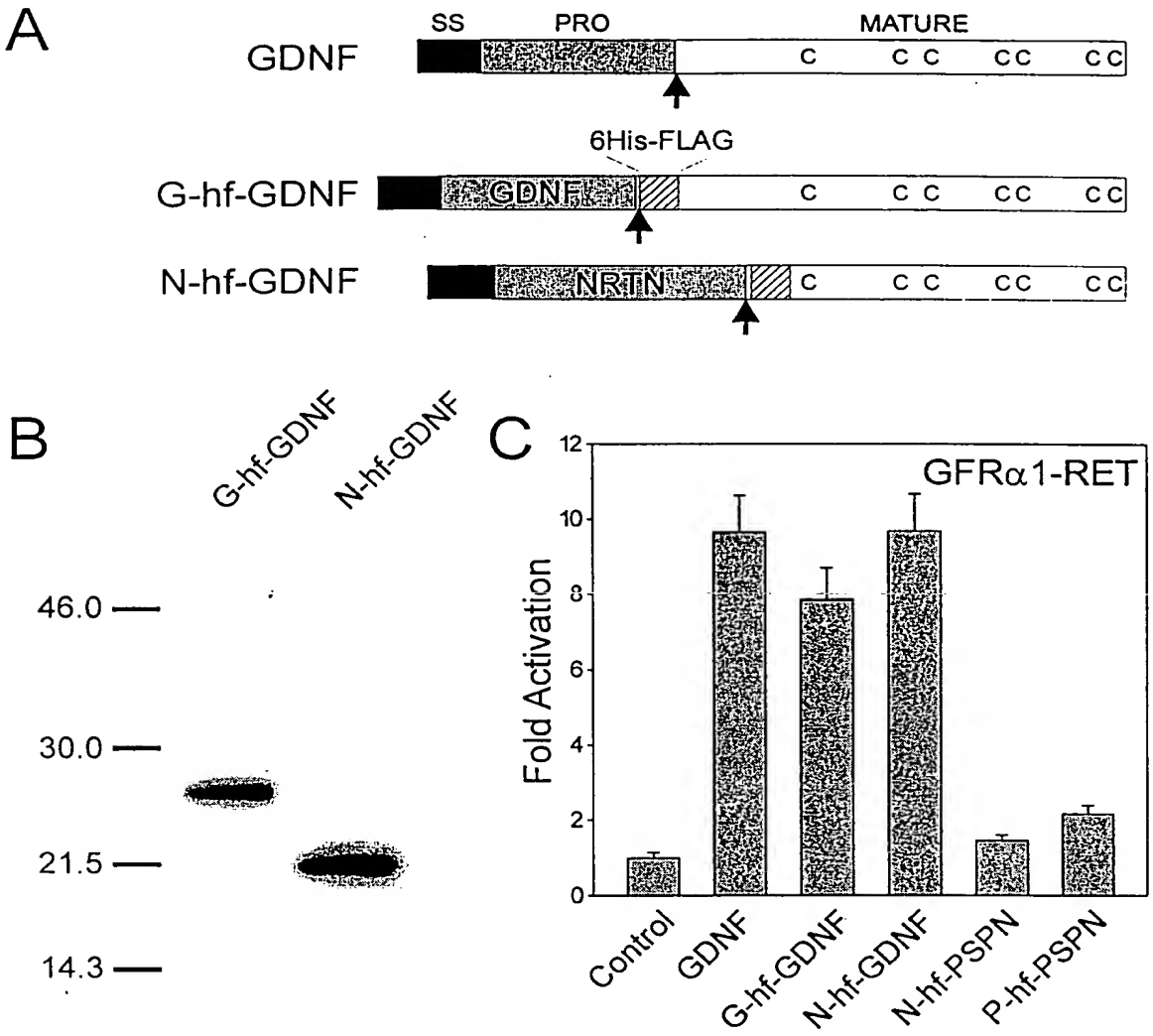
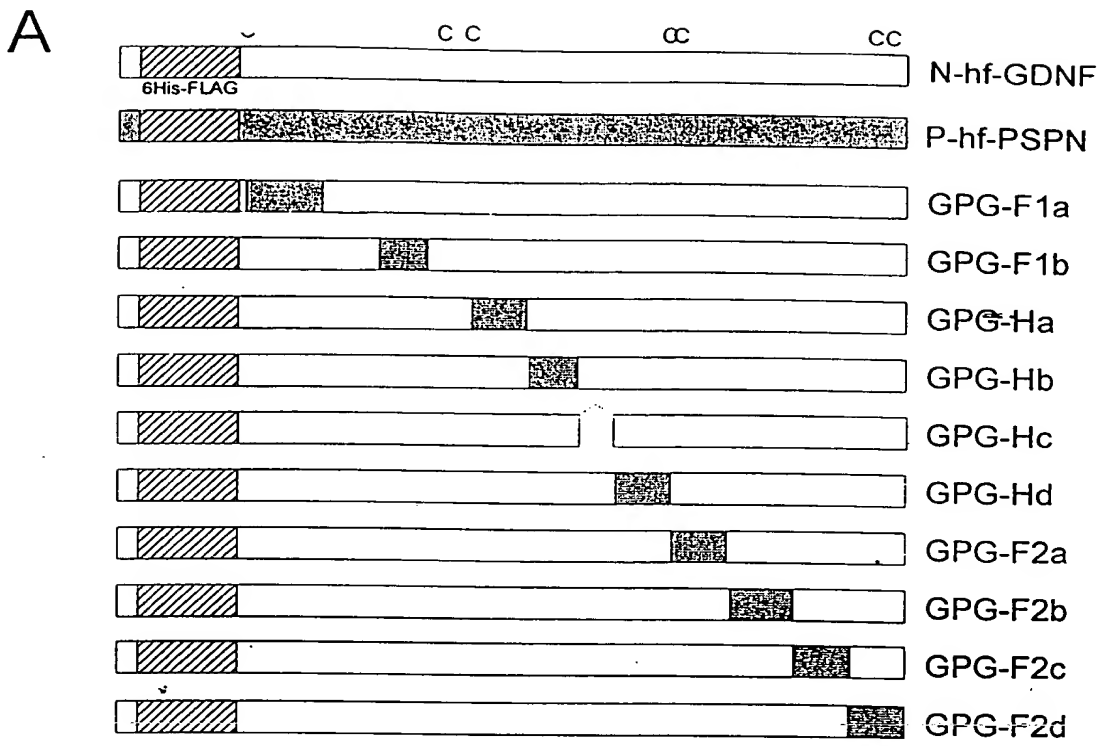

A
SS
PRO
MATURE
GDNF
C
C C
CC
CC
6His-FLAG
G-hf-GDNF
GDNF
N-hf-GDNF
NRTN
B
G-hf-GDNF
N-hf-GDNF
46.0
30.0
21.5
14.3
C
GFRα1-RET
Fold Activation
12
10
8
6
4
2
0
Control
GDNF
G-hf-GDNF
N-hf-GDNF
N-hf-PSPN
P-hf-PSPN

FIGURE 2


A
6His-FLAG
N-hf-GDNF
P-hf-PSPN
GPG-F1a
GPG-F1b
GPG-Ha
GPG-Hb
GPG-Hc
GPG-Hd
GPG-F2a
GPG-F2b
GPG-F2c
GPG-F2d

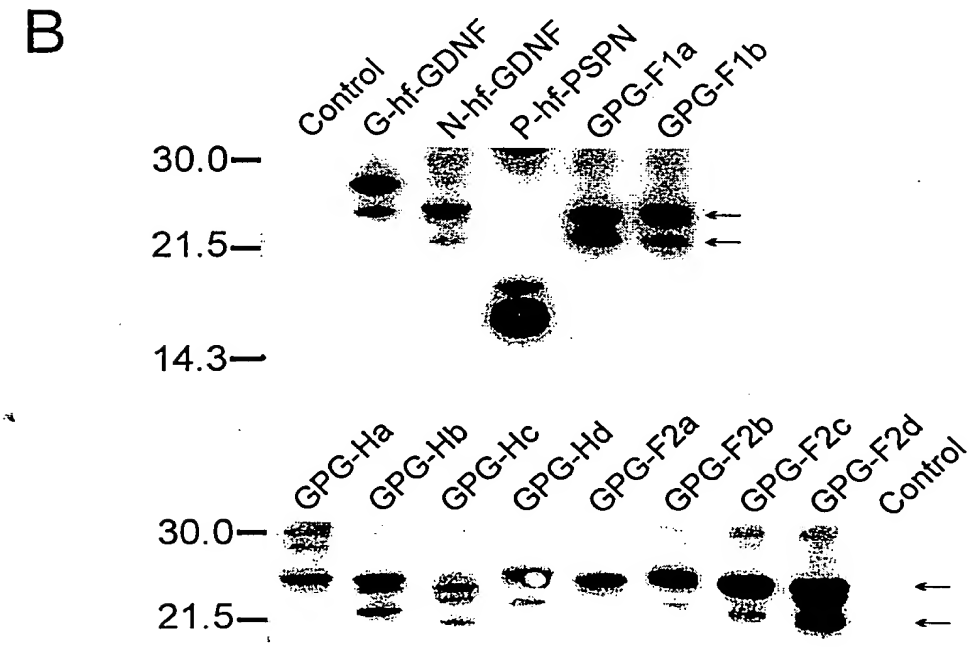

B
Control
G-hf-GDNF
N-hf-GDNF
P-hf-PSPN
GPG-F1a
GPG-F1b
30.0
21.5
14.3
GPG-Ha
GPG-Hb
GPG-Hc
GPG-Hd
GPG-F2a
GPG-F2b
GPG-F2c
GPG-F2d
Control
30.0
21.5

FIGURE 3

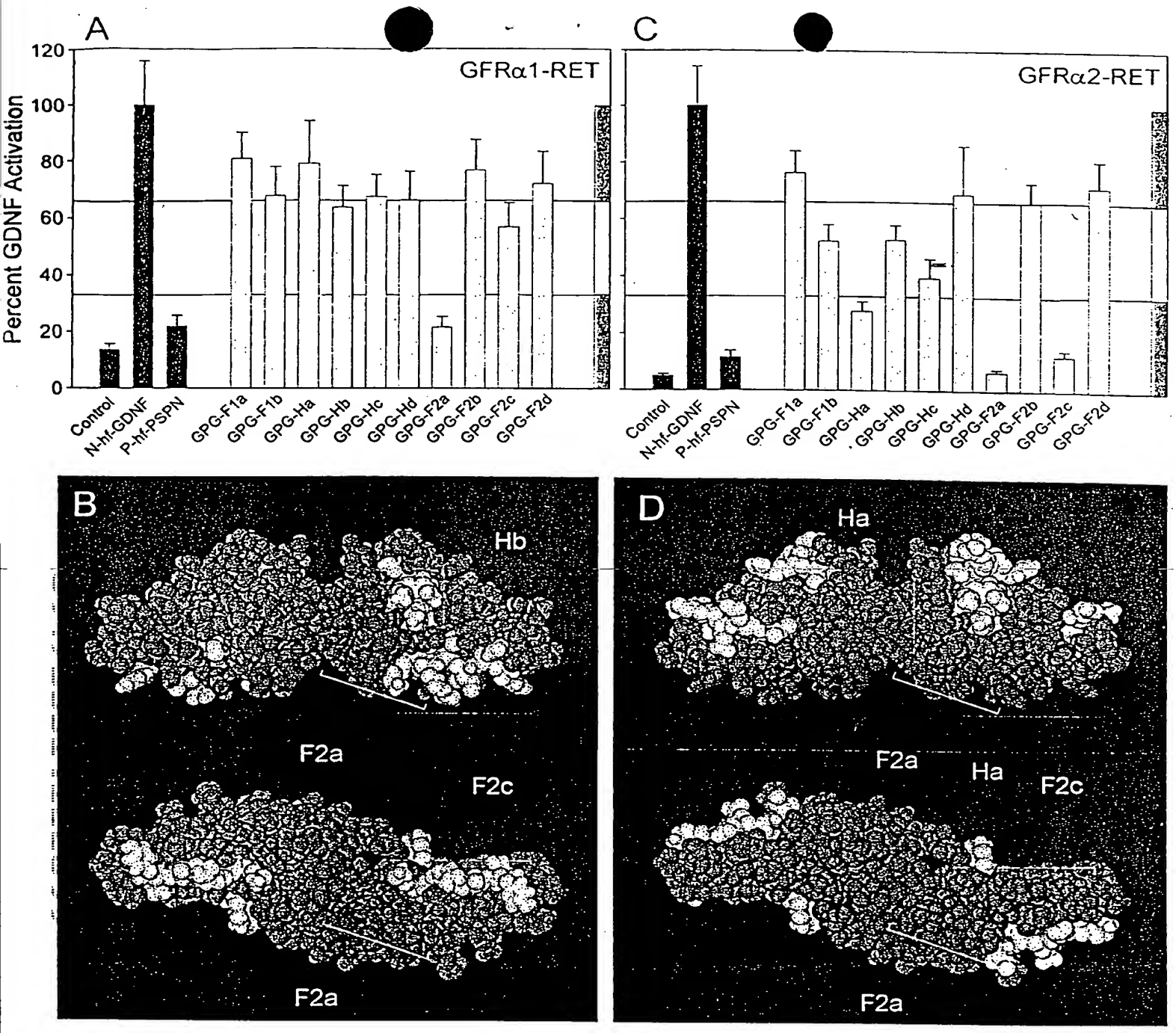

A
GFRα1-RET
Percent GDNF Activation
120
100
80
60
40
20
0
Control
N-hf-GDNF
P-hf-PSPN
GPG-F1a
GPG-F1b
GPG-Ha
GPG-Hb
GPG-Hc
GPG-Hd
GPG-F2a
GPG-F2b
GPG-F2c
GPG-F2d
C
GFRα2-RET
Control
N-hf-GDNF
P-hf-PSPN
GPG-F1a
GPG-F1b
GPG-Ha
GPG-Hb
GPG-Hc
GPG-Hd
GPG-F2a
GPG-F2b
GPG-F2c
GPG-F2d
B
Hb
F2a
F2c
F2a
D
Ha
F2a
Ha
F2c
F2a

FIGURE 4

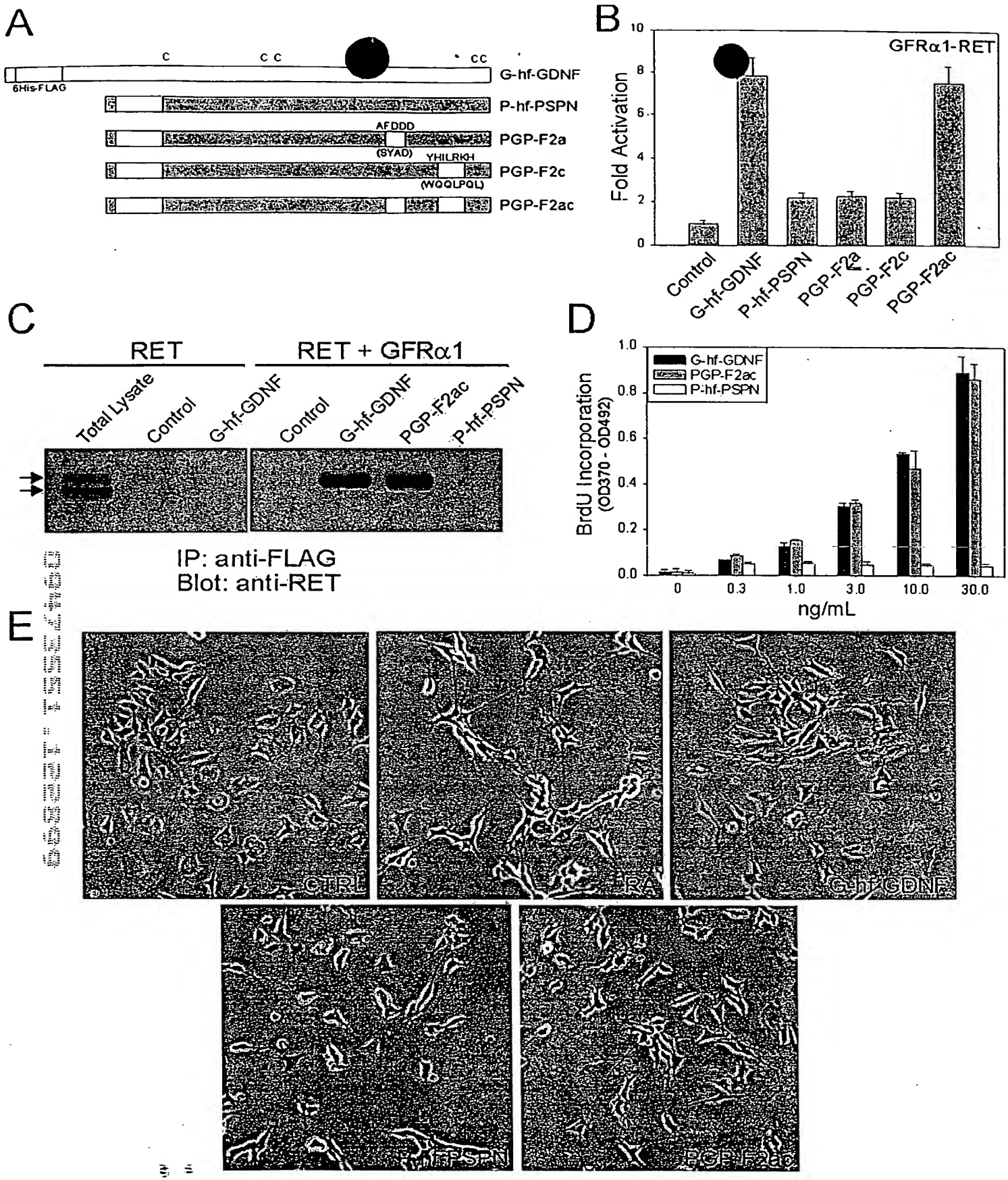

A
C
C C
CC
G-hf-GDNF
6His-FLAG
P-hf-PSPN
AFDDD
PGP-F2a
(SYAD)
YHILRKH
PGP-F2c
(WQQLPQL)
PGP-F2ac
B
GFRα1-RET
Fold Activation
10
8
6
4
2
0
Control
G-hf-GDNF
P-hf-PSPN
PGP-F2a
PGP-F2c
PGP-F2ac
C
RET
RET + GFRα1
Total Lysate
Control
G-hf-GDNF
Control
G-hf-GDNF
PGP-F2ac
P-hf-PSPN
IP: anti-FLAG
Blot: anti-RET
D
G-hf-GDNF
PGP-F2ac
P-hf-PSPN
BrdU Incorporation
(OD370 - OD492)
1.0
0.8
0.6
0.4
0.2
0.0
0
0.3
1.0
3.0
10.0
30.0
ng/mL
E
CTRL
G-hf-GDNF
P-hf-PSPN
PGP-F2ac

FIGURE 5

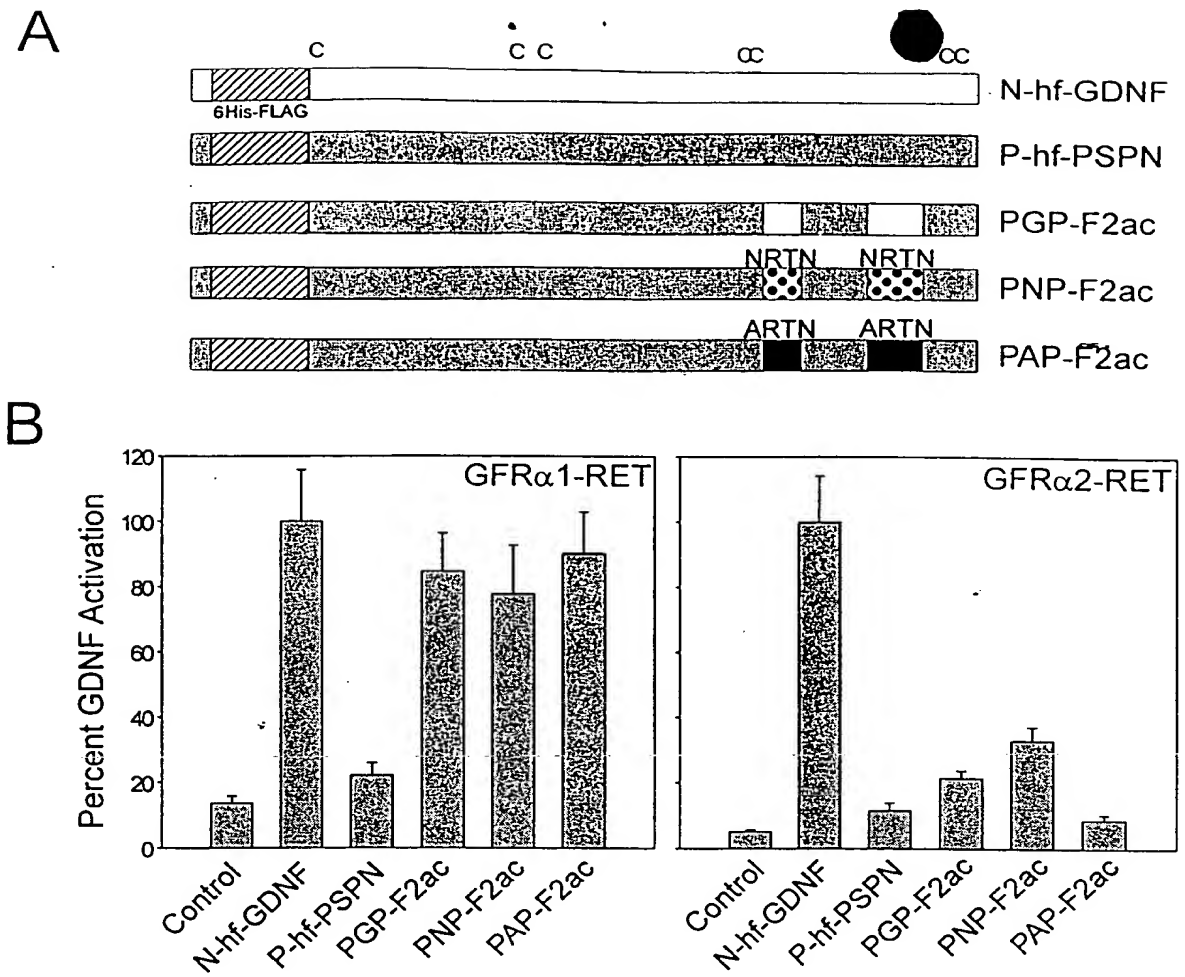

A
C
C C
CC
CC
6His-FLAG
N-hf-GDNF
P-hf-PSPN
PGP-F2ac
NRTN
NRTN
PNP-F2ac
ARTN
ARTN
PAP-F2ac
B
Percent GDNF Activation
120
100
80
60
40
20
0
GFRα1-RET
GFRα2-RET
Control
N-hf-GDNF
P-hf-PSPN
PGP-F2ac
PNP-F2ac
PAP-F2ac

FIGURE 6

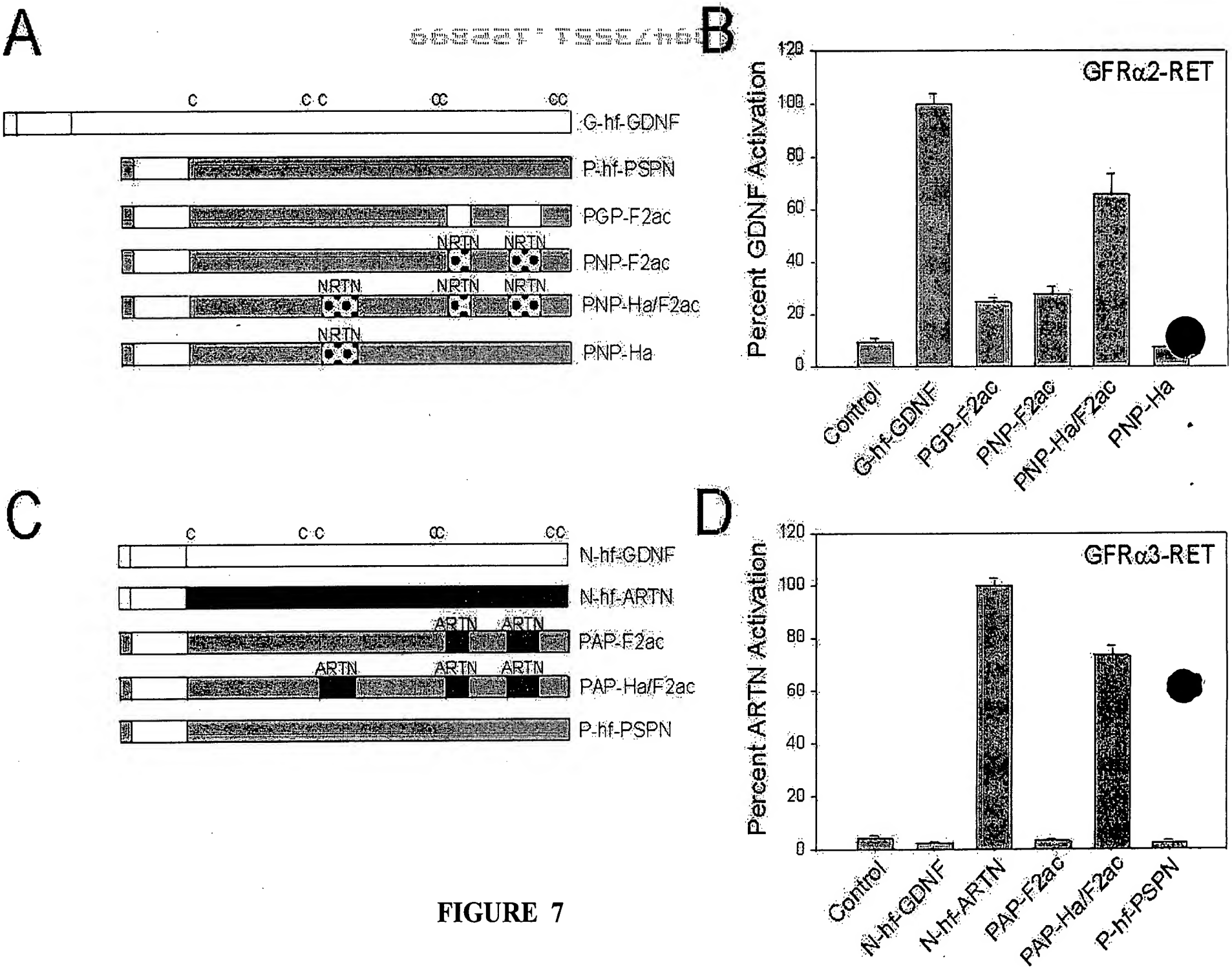

A
B
C
D
G-hf-GDNF
P-hf-PSPN
PGP-F2ac
PNP-F2ac
PNP-Ha/F2ac
PNP-Ha
NRTN
N-hf-GDNF
N-hf-ARTN
PAP-F2ac
PAP-Ha/F2ac
ARTN
GFRα2-RET
Percent GDNF Activation
Control
G-hf-GDNF
PGP-F2ac
PNP-F2ac
PNP-Ha/F2ac
PNP-Ha
GFRα3-RET
Percent ARTN Activation
N-hf-GDNF
N-hf-ARTN
PAP-F2ac
PAP-Ha/F2ac
P-hf-PSPN

FIGURE 7

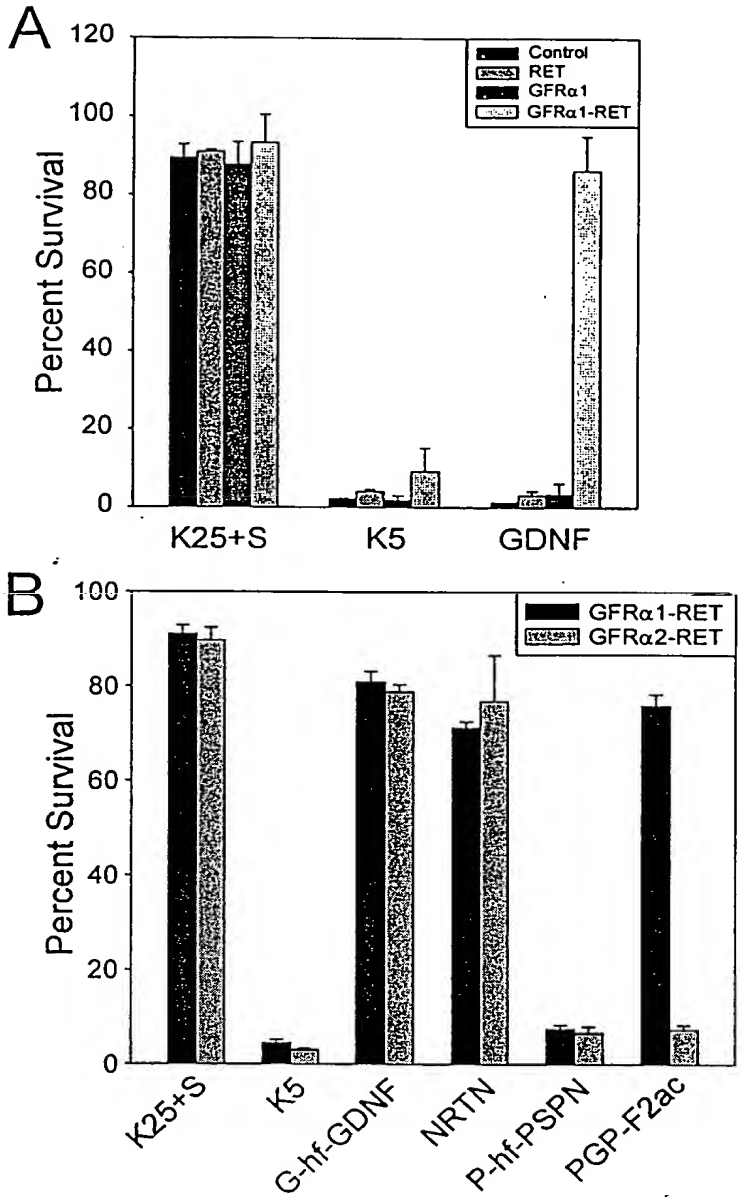

A
120
100
80
60
40
20
0
Percent Survival
Control
RET
GFRα1
GFRα1-RET
K25+S
K5
GDNF
B
100
80
60
40
20
0
Percent Survival
GFRα1-RET
GFRα2-RET
K25+S
K5
G-hf-GDNF
NRTN
P-hf-PSPN
PGP-F2ac

FIGURE 8